UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

October 23, 2007

TXR # 0054622

SUBJECT: **Mesotrione: Review of Dermal Penetration Studies (MRID 46951721, 46951722 and 46951723)**

PC Code: 122990
DP Barcode: D333418

FROM: P. V. Shah
Registration Action Branch 1 (RAB1)
Health Effects Division (7509P)

TO: Joanne Miller/James Stone (RM23)
Registration Division (7505P)

THROUGH: Dana Vogel
Branch Chief
Registration Action Branch 1
Health Effects Division (7509P)

I. CONCLUSIONS

The Registration Division (RD) requested Health Effects Division (HED) to evaluate dermal penetration studies (MRID 46951721, 46951722 and 46951723) conducted on Mesotrione. These studies were submitted by the Syngenta.

HED reviewed these studies and prepared Data Evaluation Record (DERs). The *in vivo* dermal penetration study in rats (MRID 46951723) is classified as Acceptable/Guideline study. The *in vitro* dermal penetration study through rat and human skin (MRID 46951721 and 46951722) is classified as Acceptable/Non-guideline study. Mean dermal absorption (in vivo) values ranged from 0.12-0.52% for the high dose and from 0.31-1.83% for the low dose. Based on the results of in vivo dermal absorption study, dermal absorption factor of 1% may be considered appropriate for dermal exposure assessment.

II. ACTION REQUESTED

The RD requested HED to evaluate dermal penetration studies (MRID 46951721, 46951722 and 46951723) on Mesotrione and prepare a Data Evaluation Record (DER). These studies were submitted by the Syngenta.

III STUDIES REVIEWED

1. Smith, A.D. (2004) Mesotrione: in vivo dermal penetration study in the rat using Callisto 480SC formulation A12738A. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number UR0804/REG/RE-001, Task No. T007492-03. April 30, 2004. MRID 46951723. Unpublished.

EXECUTIVE SUMMARY:

In an *in vivo* dermal absorption study (MRID No.46951723) [^{14}C]-Mesotrione (>95% radiochemical purity) was administered to a total of 32 male Wistar rats at nominal doses of 4.8 $\mu g/cm^2$ (spray dilution) or 4781 $\mu g/cm^2$ (concentrate). Four animals per exposure group were sacrificed after 10 hrs exposure. The remaining groups of 4 animals/dose were washed at 10 hours and sacrificed at 24, 72, and 120 hrs. All remaining animals were washed again at 24 and 48 hrs. Results of the study are summarized in the following table.

Dermal Absorption Rate Summary Mesotrione In Vivo Rat Dermal Absorption Study								
Dose ($\mu g/cm^2$)	**Mean percentage of Dose Absorbed & In/On Skin**							
	10 h		24 h*		72 h*		120 h*	
	Abs**	Skin***	Abs	Skin	Abs	Skin	Abs	Skin
4.8	0.31	17.62	0.61	17.60	0.96	13.51	1.83	12.60
4781	0.52	0.11	0.16	0.04	0.12	0.03	0.17	0.04

* washed at 10 hr terminated at 24, 72 and 120 hrs.
**Abs= radioactivity absorbed (sum of carcass, urine, feces GI tract and cage wash)
***Amount of radioactivity in/on skin after skin wash (stratum corneum and application site skin)

The mean total recovery of applied radioactivity ranged from 99.4–102% for all dose/duration groups. Most of the applied radioactivity was recovered in the skin washes with a mean recovery of 99 – 102% for the high dose and 79-82% for the low dose. Mean dermal absorption values ranged from 0.12-0.52% for the high dose and from 0.31-1.83 for the low dose. With the exception of the 10 hour duration, amount absorbed decreased with increased dose indicating skin is approaching saturation of penetration at higher doses. Mean radioactivity (percent of the applied dose) remaining in/on the skin ranged from 12.6 – 17.6% for the low dose and 0.03-0.11% for the high dose group. For the low dose, percent of dose remaining in/on skin decreased while percent absorbed increased with time after the 10 hour, 24 and 48 hour washes indicating that some of the material remaining in/on the skin continued to be absorbed post-wash.

This study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a dermal penetration study (870.7600) in rats.

2. Davies, D.J. (2004) Callisto 480SC formulation A12738A: *in vitro* dermal penetration of mesotrione through rat epidermis. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number

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JV1778; task number T007494-03. March 25, 2004. MRID 46951721. Unpublished.

Davies, D.J. (2004) Callisto 480SC formulation A12738A: *in vitro* dermal penetration of mesotrione through human epidermis. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number JV1777; task number T007493-03. March 25, 2004. MRID 46951722. Unpublished.

EXECUTIVE SUMMARY:

In vitro rat and human dermal absorption studies (MRID Nos.46951721 and 46951722) were conducted using glass diffusion cell *in vitro* techniques. Epidermal membranes from rat and human skin were administered [^{14}C]-mesotrione (>95.8% radiochemical purity) at doses of 4818 µg/ cm^2 (formulation concentrate) and 4.86 µg/ cm^2 (1/952 v/v aqueous spray dilution) and exposed for 10 or 24 hours post-dosing. Results of the rat and human analyses are provided in Tables 1 and 2.

Table 1. Dermal Absorption Summary – Absorbed Mesotrione Through Rat Epidermis

Dose (µg/cm^2)	Mean percentage of Dose Absorbed & In Epidermis			
	10 hrs		24 hrs	
	Receptor Fluid	Epidermis	Receptor Fluid	Epidermis
4.86	6.78	17.6	6.59	20.9
4818	0.05	0.36	0.05	0.32

Rat Epidermis

The mean total recovery of applied radioactivity ranged from 98.6–103% for all dose/duration groups. Most of the applied radioactivity was recovered in the skin washes with a mean recovery of 98.5 – 103%. The mean percentage of absorbed radioactivity in the receptor fluid ranged from 0.05 to 6.78% at 10 hours and from 0.05 to 6.59% at 24 hours. Mean amount of applied dose remaining in the rat epidermis for the 10 hour exposure was 0.36% for the high dose and 17.6% for the low dose. For the 24 hour exposure period, mean percent of applied dose remaining in the epidermis was 0.32% for the high dose and 20.9% for the low dose.

Table 2. Dermal Absorption Summary – Absorbed Mesotrione Through Human Epidermis

Dose (µg/cm^2)	Mean percentage of Dose Absorbed & In/On Epidermis					
	10 hrs			24 hrs		
	Receptor Fluid	Stratum Corneum	Remaining Epidermis	Receptor Fluid	Stratum Corneum	Remaining Epidermis
4.86	0.15	0.29	1.71	0.20	0.29	1.83
4818	0.04	0.09	0.06	0.04	0.09	0.02

Human Epidermis

The mean total recovery of applied radioactivity ranged from 94.6-102% for all dose/duration groups. Most of the applied radioactivity was recovered in the skin washes with a mean recovery of 91.9-102%. Mean percent of dose in the receptor fluid ranged from 0.04-0.20%.

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Mean amount of applied dose remaining in the stratum corneum was 0.09% for the high dose and 0.29% for the low dose for the both exposure periods. Mean amount in the remaining epidermis for the 10 hour exposure was 1.71% for the low dose and 0.06% for the high dose. For the 24 hour exposure, mean percent of dose in the epidermis was 1.83% for the low dose and 0.02% for the high dose.

The *in vitro* studies on rat and human skin are Acceptable/Nonguideline supplement and provide supplemental data to the guideline requirement for a dermal penetration study (870.7600).

Attachments: Hard Copy of 46951723.der and 46951721.der

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DATA EVALUATION RECORD

MESOTRIONE

STUDY TYPE: DERMAL PENETRATION STUDY–RAT
[OPPTS: 870.7600 (§85-2)]
MRID 46951723

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 160B-2007

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

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Date: 10/30/07

Template version 02/06

TXR#: 0054622

DATA EVALUATION RECORD

STUDY TYPE: Rodent *In Vivo* Dermal Penetration Study – Rat [OPPTS 870.7600 ['85-2]; OECD none

PC CODE: 122990 **DP BARCODE:** 333418
DECISION: 371185

TEST MATERIAL (PURITY): [^{14}C]-Mesotrione (>95% radiochemicalpurity)

SYNONYMS: 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Smith, A.D. (2004) Mesotrione: *in* vivo dermal penetration study in the rat using Callisto 480SC formulation A12738A. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number UR0804/REG/RE-001, Task No. T007492-03. April 30, 2004. MRID 46951723. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419-8300

EXECUTIVE SUMMARY:

In an *in vivo* dermal absorption study (MRID No.46951723) [^{14}C]-Mesotrione (>95% radiochemical purity) was administered to a total of 32 male Wistar rats at nominal doses of 4.8 µg/cm^2 (spray dilution) or 4781 µg/cm^2 (concentrate). Four animals per exposure group were sacrificed after 10 hrs exposure. The remaining groups of 4 animals/dose were washed at 10 hours and sacrificed at 24, 72, and 120 hrs. All remaining animals were washed again at 24 and 48 hrs. Results of the study are summarized in the following table.

Dermal Absorption Rate Summary Mesotrione In Vivo Rat Dermal Absorption Study

Dose (µg/cm^2)	Mean percentage of Dose Absorbed & In/On Skin							
	10 h		24 h*		72 h*		120 h*	
	Abs**	Skin***	Abs	Skin	Abs	Skin	Abs	Skin
4.8	0.31	17.62	0.61	17.60	0.96	13.51	1.83	12.60
4781	0.52	0.11	0.16	0.04	0.12	0.03	0.17	0.04

* washed at 10 hr terminated at 24, 72 and 120 hrs.
**Abs= radioactivity absorbed (sum of carcass, urine, feces GI tract and cage wash)
***Amount of radioactivity in/on skin after skin wash (stratum corneum and application site skin)

The mean total recovery of applied radioactivity ranged from 99.4–102% for all dose/duration groups. Most of the applied radioactivity was recovered in the skin washes with a mean recovery of 99 – 102% for the high dose and 79-82% for the low dose. Mean dermal absorption values ranged from 0.12-0.52% for the high dose and from 0.31-1.83 for the low dose. With the exception of the 10 hour duration, amount absorbed decreased with increased dose indicating skin is approaching saturation of penetration at higher doses. Mean radioactivity (percent of the applied dose) remaining in/on the skin ranged from 12.6 – 17.6% for the low dose and 0.03-0.11% for the high dose group. For the low dose, percent of dose remaining in/on skin decreased while percent absorbed increased with time after the 10 hour, 24 and 48 hour washes indicating that some of the material remaining in/on the skin continued to be absorbed post-wash.

This study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a dermal penetration study (870.7600) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Note: Results of *in vitro* rat and human dermal absorption studies are provided for comparison purposes.

Dermal Absorption Summary – Absorbed Mesotrione Through Rat Epidermis MRID 46951721				
Dose ($\mu g/cm^2$)	**Mean percentage of Dose Absorbed & In Epidermis**			
	10 hrs		24 hrs	
	Receptor Fluid	Epidermis	Receptor Fluid	Epidermis
4.86	6.78	17.6	6.59	20.9
4818	0.05	0.36	0.05	0.32

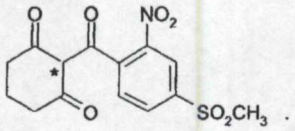
Dermal Absorption Summary – Absorbed Mesotrione Through Human Epidermis MRID 46951722						
Dose ($\mu g/cm^2$)	**Mean percentage of Dose Absorbed & In/On Epidermis**					
	10 hrs			24 hrs		
	Receptor Fluid	Stratum Corneum	Remaining Epidermis	Receptor Fluid	Stratum Corneum	Remaining Epidermis
4.86	0.15	0.29	1.71	0.20	0.29	1.83
4818	0.04	0.09	0.06	0.04	0.09	0.02

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

[^{14}C]-Mesotrione

Description:	Light beige, solid
Lot/batch #:	Reference No. Y11339/502
Purity:	>95% radiochemicalpurity
Compound stability:	Not available
CAS # for TGAI:	Not available
Structure:	
Vehicle/Solvent used:	Distilled water
Radiolabelling:	Indicated in structure by asterisk
Specific Activity:	1.36 GBq/mM (4.0 MBq/mg)
Radiochemical Purity:	>95%
Source:	Syngenta Crop Protection Inc.
Other comments:	None

2. Relevance of test material to proposed formulation(s): Not relevant

3. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Age/weight at study initiation:	6-8 weeks/223-250g
Source:	Biological Services Section, Alderley Park, Macclesfield, Chesire
Housing:	By groups in stock cages
Diet:	Rat and mouse maintenance diet, Special Diets Services, Stepfield, Witham , Essex; *ad libitum*
Water:	Tap; *ad libitum*
Environmental conditions:	**Temperature:** 22±3°C **Humidity:** 30-70% **Air changes:** ≥15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	6 days in individual metabolism cages prior to dosing

B. STUDY DESIGN:

1. Dose:

Rationale: Dose selection was based on direct exposure to formulation concentrate or anticipated dermal deposition of recommended field dilution.

Nominal doses: 4.8 and 0.005 mg ai/cm^2 skin.

Actual doses: 4.78 and 0.0048 mg ai/rat (10 cm^2 skin; see Table 1 for details). Actual doses were calculated as the mean dose applied to each of 16 animals/dose rate as determined by radiochemical analysis of the two dosing solutions before, during, and after dosing. These values were also used to assess the homogeneity of each dose.

Dose volume: 10µL/cm^2 skin

Duration of exposures (time from dose to skin wash): 10 hrs.

Termination periods (time from dose to sacrifice): All animals were washed after 10 hrs exposure at each dose rate. Four animals per exposure group were sacrificed after 10 hrs exposure and the remaining groups of 4 animals/dose were sacrificed at 24, 72, and 120 hrs. All remaining animals were again washed at the 24 hr termination period and those remaining following the 24 hr wash were washed again at 48 hrs. For the animals exposed to the formulation concentrate, two animals died after the 10 hr exposure period and one animal exhibited low recovery of radioactivity. These animals were excluded leaving only 3 animals /group at the 24, 72, and 120 hr termination periods. No animals were excluded from the 1/952 dilution of the formulation concentrate dose group leaving four animals per group at each termination period.

Number of animals/group: 4 animals/dose/exposure period.

2. **Animal preparation:** On the day before dosing, the area behind the shoulders of each animal was clipped and the clipped area swabbed with acetone. Care was taken not to abrade the skin or non-abraded areas were selected for application. The site of application (approximately 10 cm^2) was defined and protected by gluing a silicone O-ring to the unabraded skin using a cyanoacrylate-based glue. To protect the application site a strip of non-occlusive elasticized bandage was placed around the rat and over the application device. Animals were acclimatized individually by placing them in metabolism cages for at least 12 hours prior to dosing.

3. **Dose preparation, administration and quantification:**

Preparation: Dosing suspensions were prepared on the day prior to dosing. The formulation concentrate was prepared by mixing sufficient milled recrystallized technical grade unlabelled mesotrione and [^{14}C]-mesotrione in an appropriate amount of blank formulation (CTL test substance reference number Y11339/503) to yield 48.0mg a.i. per 100 µL dose volume. The 1/952 aqueous dilution (spray formulation) of the formulation concentrate (mesotrione formulation A12738A) was prepared by mixing the appropriate amount of [^{14}C]-mesotrione in an appropriate volume of the blank formulation, adjuvant (Agral 90) and distilled water to yield 0.050 mg a.i. per 100 µL dose volume.

Application: The dosing solutions were stirred continuously during dosing. The required volume (100µL/rat) of the formulation concentrate or the 1/952 dilution was applied and spread evenly across the surface of the skin site using a positive displacement pipette. The dosing site was covered by a charcoal filter and a nylon gauze pad which were placed over the cemented o-ring and held in place by a non-occlusive elasticized bandage. Sixteen rats were dosed per exposure concentration. The pipette tip used to apply the dose was retained for subsequent extraction of residual activity for calculation of the actual dose applied. Dosed animals were returned to their metabolism cages equipped for collection of urine and feces. Provision was not made for CO_2 collection.

Quantification: Radioactivity (dpm) and radiochemical purity were determined before dosing. Homogeneity of the doses was determined from radiochemical analysis of dose-size aliquots of the dosing solutions taken before, during and after dosing. Pre-dose and post-dose aliquots were analyzed to confirm radioactivity levels and homogeneity. The syringe tips used for each dose were analyzed for radioactivity and the individual doses were corrected for radioactivity retained in the dosing syringe tips. Data are summarized in Table 1.

TABLE 1. Dosing

Formulation	Nominal dose (mg/cm^2)	Actual dose (mg/cm^2)	Specific activity (MBq/mg)
Concentrate	4.80	4.781	0.0095
1/952 v/v	0.0050	0.00480	4.018

4. **Skin wash (pre-sacrifice):** Animals sacrificed after 10 hours exposure were processed according to the terminal procedure. Application sites of the remaining animals (12 per dose) were washed. Bandages, gauze covers, and charcoal filters were removed and retained for analysis. Each application site was washed using 10 sponges pre-wetted with a 3% dishwashing liquid (Dove; Lever Brothers Co.) and 10 sponges wetted with water. Each application site was then dry-sponged twice to remove residual water. All sponges for each animal were combined and retained for radiochemical analysis. A fresh gauze cover and a new elasticized bandage was fitted to each animal and each was returned to its metabolism cage. Any excreta collected during the procedure were combined with the 0-10 hour urine and feces samples collected immediately after skin washing. Rats retained after the 24 hour sacrifice period were washed again at 48 hours post-dosing. The elasticized bandages were removed and combined with those from the previous washing for each rat. The gauze covers were removed and the application sites washed with 4 sponges pre-wetted with 3% Dove dishwashing detergent, 3 sponges wetted with distilled water and two dry sponges. Combined sponges for each rat were retained, a new gauze cover was fitted, a new elasticized bandage was fitted, and the rats were returned to their respective metabolism cages. .

5. **Sample collection:** Urine and feces were collected from each group of four animals for each time point (10, 24, 48, 72, 96 and 120 hr) and each dosing level. At each termination period 4 rats were taken per dose level and lightly anaesthetized using halothane vapor. The protective cover, gauze, and filter were removed and saved for each rat. Residual dosing preparation was removed by washing the skin with 10 sponges of 3% dishwashing detergent, 10 sponges with distilled water and 2 dry sponges. Sponges from each animal were combined and saved. Animals were exsanguinated under full anaesthesia and blood was collected into two heparinized tubes. One of the heparinized blood sampes was centrifuged to separate plasma. The O-ring and annular ring of untreated skin were excised and pinned on a board. After O-ring removal the treated area and annular ring area was washed and dried and the application site was tape-stripped to remove the stratum corneum and expose the epidermis. The tape strip and residual skin were saved in separate containers. Residual urine was collected from the urinary bladder and added to the urine sample. The gastrointestinal

tract and contents was removed from each animal. All samples except whole blood were saved with the carcass and stored frozen. Whole blood was refrigerated. Following collection of excreta, metabolism cages were washed with 1% sodium bicarbonate (10 mg/mL) followed by a wash with 0.5M HCl. Washings were stored under refrigeration until analyzed. Individual organs and other tissues were not analyzed separately.

6. **Sample preparation and analysis:** Details of sample preparation are provided in Table 2.

TABLE 2. Sample preparation details

Sample media	Preparation details
Skin wash sponges and tape strips with attached stratum corneum	Sponges used for skin washings and adhesive tape strips used to remove the stratum corneum were solubilized in Soluene tissue digestant. Duplicate aliquots were mixed with scintillant and counted directly by liquid scintillation counting.
Feces	Mixed with distilled water and homogenized to a paste. Small samples were oxidized by combustion and the $^{14}CO_2$ generated was absorbed and mixed with scintillant prior to analysis by liquid scintillation counting.
Urine	Duplicate aliquots of urine were added to scintillant and analyzed by liquid scintillation counting.
Blood (whole and plasma)	Plasma was added directly to scintillant and analyzed by liquid scintillation counting. Whole blood was solubilized in Soluene and aliquots were counted by liquid scintillation counting.
Cage wash	Duplicate aliquots of cage washings were added directly to scintillant and analyzed by liquid scintillation counting.
Elasticized bandages	Extracted with acetonitrile. Duplicate aliquots of the extractant were added to scintillant and analyzed by liquid scintillation counting
Gastrointestinal tract including contents	Solubilized in Soluene tissue digestant and analyzed by liquid scintillation counting.
Carcass	Solubilized in tissue digestant at 40EC and analyzed by liquid scintillation counting.
Nonocclusive cover (i.e. nylon pad and/or charcoal filter)	Extracted with acetonitrile. Duplicate aliquots of the extractant were added to scintillant and analyzed by liquid scintillation counting.
O-ring (enclosure)	Extracted with acetonitrile. Duplicate aliquots of the extractant were added to scintillant and analyzed by liquid scintillation counting.
Skin at application site	Skin was solubilized in Soluene tissue digestant and analyzed by liquid scintillation counting.
Pipette tip (also used as spreader)	Extracted with acetonitrile and extractant was analyzed by liquid scintillation counting.

Liquid scintillation counting results (cpm) were converted to dpm using external standardization and appropriate quench correction data entered into the instrument computer of a Packard Tricarb Liquid Scintillation Counter (LSC). Oxidation efficiency was determined by comparison of sample results to counting efficiencies obtained from oxidation of tissues spiked with known standards of [^{14}C]-Mesotrione. All samples were prepared and counted in duplicate.

Limits of detection (LOD) for the formulation dose samples were determined to be 0.0357 and 0.0624 µg equivalents of mesotrione for direct LSC and oxidized samples.respectively. The LOD for the 1/952 dilution were determined to be 0.0001 and 0.0001 µg equivalent mesotrione for direct LSC and oxidized samples, respectively.

Analytical controls were only used to validate counting procedures for sample oxidation only.

Total amounts of radioactivity in samples were reported both as µg equivalents of mesotrione and as percentages of the applied dose.

II. RESULTS:

A. SIGNS AND SYMPTOMS OF TOXICITY:

After 10 hours exposure to mesotrione formulation concentrate, all the rats appeared to be subdued and were cold to the touch. The rats appeared normal during the first sampling interval (10 hrs). Two rats died before the 24 hour sampling interval. These deaths were attributed to stress rather than overt toxicity.

B. SUMMARY TABLES:

TABLE 3: Amount of mesotrione in each matrix at specified hours post-application of formulation concentrate at 4.78 mg/cm^2 (skin wash at 10 hours)

Matrix analyzed	Residues in matrix (% of applied dose)			
	10 hr (4rats)	24 hr (3 rats)	72 hr (3 rats)	120 hr (3 rats)
10 hr skin wash	NA	100.38±2.05	99.06±4.51	101.60±1.35
48 hr skin wash	NA	NA	0.07±0.02	0.05±0.02
Terminal skin wash	101.00±1.60	0.08±0.03	0.03±0.01	0.02±0.01
O-rings	0.02±0.01	0.05±0.05	0.07±0.08	0.01±0.01
Covers	0.03±0.05	0.44±0.51	<0.04	0.08±0.07
Stratum corneum	0.05±0.02	0.03±0.02	0.02±0.01	0.03±0.01
Application site skin	0.06±0.09	0.01±0.01	0.01±<0.01	0.01±<0.01
Urine	<0.04	<0.04	<0.04	0.01
Feces	<0.06	<0.06	0.02	0.04±0.01
Cage wash	<0.04	<0.04	0.01	0.03±0.01
Terminal cage wash	<0.04	<0.04	<0.04	<0.04
Bandage	0.01±<0.01	0.03±0.02	0.01	0.02±0.01
Carcass + blood	0.49±0.80	0.11±0.04	0.10	0.10
GI tract + contents	0.01±<0.01	0.01±<0.01	0.01	0.01
Total potentially absorbable	0.10±0.12	0.04±0.01	0.02±0.01	0.03±0.02
Total absorbed	0.52±0.80	0.16±0.06	0.12±0.07	0.17±0.09
Total recovered	101.68±1.69	101.15±2.01	99.41±4.42	102.00±1.35

Data taken from Table 2, pg. 38; MRID 46951723.

TABLE 4: Amount of mesotrione in each matrix at specified hours post-application of a 1/952 aqueous dilution of formulation concentrate at 0.0048 mg/cm^2 (skin wash at 10 hours)

Matrix analyzed	Residues in matrix (% of applied dose)[1]			
	10 hr (4 rats)	24 hr (4 rats)	72 hr (4 rats)	120 hr (4 rats)
10 hr skin wash	NA	79.14±4.83	80.31±4.28	81.55±3.02
48 hr skin wash	NA	NA	2.19±1.13	2.45±0.86
Terminal skin wash	81.31±5.83	3.37±1.27	1.44±0.67	1.16±1.27
O-rings	0.61±0.83	0.15±0.09	0.14±0.06	0.07±0.02
Covers	0.08±0.13	0.32±0.24	0.91±1.54	0.07±0.07
Stratum corneum	16.89±5.42	17.14±5.40	13.30±3.63	12.44±1.55
Application site skin	0.73±0.40	0.46±0.32	0.21±0.11	0.16±0.05
Urine	0.01±<0.001	0.02±0.01	0.05±0.05	0.07±0.04
Feces	<0.0001	<0.0001	0.15±0.24	0.49±0.34
Cage wash	0.01±<0.001	0.05±0.06	0.08±0.07	0.16±0.07
Terminal cage wash	0.02±0.02	0.02±0.01	0.02±<0.001	0.03±0.02
Bandage	0.02±0.00	0.09±0.09	0.09±0.05	0.16±0.12
Carcass + blood	0.23±0.06	0.36±0.20	0.55±0.65	0.86±0.48
GI tract + contents	0.02±<0.001	0.07±0.09	0.03±0.01	0.05±0.04
Total potentially absorbable	17.62±5.70	17.60±5.09	13.51±3.63	12.60±1.52
Total absorbed	0.31±0.05	0.61±0.28	0.96±1.05	1.83±0.90
Total recovered	99.92±1.76	101.20±1.48	99.47±2.29	99.73±1.06

Data taken from Table 4, pg. 40; MRID 46951723.

C. TOTAL ABSORBED DOSE:

The total absorbed dose was calculated as the sum of the total radioactivity recovered from urine, feces, bandages, cage washes, carcass + blood, and gastrointestinal tract and contents. Radioactivity found in bandages was attributed to contamination with urine and feces and was, therefore, attributable to absorption.

Results of analysis are summarized in Tables 3, and 4. Recovery of the applied dose (material balance) was acceptable (group means ranged from 99.4-102.0% for the formulation concentrate and from 99.5-101.2% for the spray dilution of the formulation concentrate). Results were adjusted for incomplete recovery of the applied dose in one animal with low recovery (77.5%) and two animals that died. Values at the limit of detection were included in the calculations and values <LOD were raised to the LOD for calculation of group means.

The majority of the administered dose was recovered from skin washings, O-rings, and covers. 0.16-0.73% of the applied dose was retained at the application site for the formulation concentrate and 0.01-0.06% for the spray dilution of the formulation concentrate. Estimates of dermal absorption were based on the sum of residues recovered in urine (including cage wash and rinse) + feces + carcass + bandage + blood + gastrointestinal tract and contents. The study authors exclude residues retained at the skin site in estimates of dermal absorption because the values decline over time and could mask any increase of residues in the rest of the body over the observation period.

Given the uncertainty regarding actual deposition under actual field conditions, it is considered appropriate to derive an estimate of dermal absorption based on the results from

the low dose group (0.0048 mg/cm^2), as percent dermal absorption was greatest at this dose level. Based on the likely worker exposure time frame, it is considered most appropriate to adopt the dermal absorption value calculated for the group of animals for which the skin site was washed after 10 hours, and which were sacrificed after 120 hrs. Mean dermal absorption for this group of animals was 1.83%. This value does not include the potentially absorbable radioactivity present in the stratum corneum (12.44% of applied) and the skin at the application site (0.16%). The radioactivity retained by the skin after 120 hours post-treatment is not considered to be further absorbed. The radioactivity present in the stratum corneum after 120 hours post treatment is considered not to be available for further absorption but to be labile to loss by desquamation.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

Following a 10 hr *in vivo* dermal exposure to mesotrione formulation concentrate (A12738A) the applied dose was almost completely removed by a mild skin washing with 3% dishwashing detergent. Absorption accounted for <0.2% of the applied dose, including epidermal residues, following a recovery period of 5 days.

Following a 10 hr *in vivo* dermal exposure to a 1/952 dilution (spray strength) of the formulation concentrate, absorption accounted for 1.8%, of the applied radioactivity with an additional 0.16% of the applied dose present in the epidermis after 120 hours. When the data were applied to an absorption model, absorption was consistently low, but increased rapidly and reached a plateau within the 10 hour exposure period. Radioactivity in urine and feces was at or below the limit of detection, so no further increase on absorption of mesotrione was observed beyond the 10 hour exposure period. After 10 hours exposure to mesotrione, 16.9% of the administered dose remained in the stratum corneum, decreasing to 12.4% of the recovered radioactivity after 120 hrs, indicating that further absorption from the stratum corneum was unlikely. Using the exponential saturation model, the maximum absorption of mesotrione through skin was estimated to be 1%.

The absorption data for the formulation concentrate and spray strength dilution indicated that mesotrione was very poorly absorbed through rat skin *in vivo*.

B. REVIEWER COMMENTS:

Total dermal exposure at the 10 hour sacrifice and skin wash was 0.52 and 0.31%, respectively, for the formulation concentrate and 1/952 spray dilution. The skin at the application site for all test animals was washed after 10 hours of exposure. The 10 hr exposure time was chosen based on likely human exposure duration. The total absorbed values for the 24, 72, and 120 hour post-treatment sacrifice periods for both dose levels are given in Tables 3 and 4.

The estimated total absorbed is the sum of the total radioactivity recovered from the washed skin at the application site, urine, feces, bandages, cage washes, carcass + blood, and gastrointestinal tract and contents. This value is considered conservative as a portion of the

active ingredient was retained at the skin site and it is unlikely that all of the skin residues will become systemically available. The study authors chose not to include the estimated value for each observation period at each dose, but rather chose to report total absorbed dose which is the estimated value minus the percent of applied dose remaining in the dermal layer at each observation period. Because the value for radioactivity retained at the skin application site is low at each observation period for each dose, there is little effect on the outcome of the study whether or not the estimated value is considered.

This study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a dermal penetration study (870.7600) in rats.

C. STUDY DEFICIENCIES:

Some liquid scintillation procedures and sample preparation methods were incompletely described. Because material balances were high for both dose rates at each sampling interval, these omissions did not appear to affect the results of this dermal penetration study in rats. Likewise, reporting total absorbed dose rather than the estimated absorbed dose did not appear to affect the results of this dermal penetration study.

DATA EVALUATION RECORD

MESOTRIONE

STUDY TYPE: DERMAL PENETRATION STUDY–RAT/HUMAN
[OPPTS: 870.7600 (§85-2) supplement]
MRID 46951721, 46951722

Prepared for

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Disclaimer

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Registration Action Branch 1, Health Effects Division (7509P) **Date:** 10/30/07

Template version 02/06

TXR#: 0054622

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Dermal Penetration Study – Rat/Human [OPPTS 870.7600 [§ 85-2]; OECD none

PC CODE: 122990 **DP BARCODE:** 333418
DECISION: 371185

TEST MATERIAL (PURITY): [^{14}C]-Mesotrione (>95% radiochemicalpurity)

SYNONYMS: 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Davies, D.J. (2004) Callisto 480SC formulation A12738A: *in vitro* dermal penetration of mesotrione through rat epidermis. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number JV1778; task number T007494-03. March 25, 2004. MRID 46951721. Unpublished.

Davies, D.J. (2004) Callisto 480SC formulation A12738A: *in vitro* dermal penetration of mesotrione through human epidermis. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory report number JV1777; task number T007493-03. March 25, 2004. MRID 46951722. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419-8300

EXECUTIVE SUMMARY:

In vitro rat and human dermal absorption studies (MRID Nos.46951721 and 46951722) were conducted using glass diffusion cell *in vitro* techniques. Epidermal membranes from rat and human skin were administered [^{14}C]-mesotrione (>95.8% radiochemical purity) at doses of 4818 µg/ cm^2 (formulation concentrate) and 4.86 µg/ cm^2 (1/952 v/v aqueous spray dilution) and exposed for 10 or 24 hours post-dosing. Results of the rat and human analyses are provided in Tables 1 and 2.

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Table 1. Dermal Absorption Summary – Absorbed Mesotrione Through Rat Epidermis				
Dose (µg/cm²)	Mean percentage of Dose Absorbed & In Epidermis			
	10 hrs		24 hrs	
	Receptor Fluid	Epidermis	Receptor Fluid	Epidermis
4.86	6.78	17.6	6.59	20.9
4818	0.05	0.36	0.05	0.32

Rat Epidermis

The mean total recovery of applied radioactivity ranged from 98.6–103% for all dose/duration groups. Most of the applied radioactivity was recovered in the skin washes with a mean recovery of 98.5 – 103%. The mean percentage of absorbed radioactivity in the receptor fluid ranged from 0.05 to 6.78% at 10 hours and from 0.05 to 6.59% at 24 hours. Mean amount of applied dose remaining in the rat epidermis for the 10 hour exposure was 0.36% for the high dose and 17.6% for the low dose. For the 24 hour exposure period, mean percent of applied dose remaining in the epidermis was 0.32% for the high dose and 20.9% for the low dose.

Table 2. Dermal Absorption Summary – Absorbed Mesotrione Through Human Epidermis						
Dose (µg/cm²)	Mean percentage of Dose Absorbed & In/On Epidermis					
	10 hrs			24 hrs		
	Receptor Fluid	Stratum Corneum	Remaining Epidermis	Receptor Fluid	Stratum Corneum	Remaining Epidermis
4.86	0.15	0.29	1.71	0.20	0.29	1.83
4818	0.04	0.09	0.06	0.04	0.09	0.02

Human Epidermis

The mean total recovery of applied radioactivity ranged from 94.6-102% for all dose/duration groups. Most of the applied radioactivity was recovered in the skin washes with a mean recovery of 91.9-102%. Mean percent of dose in the receptor fluid ranged from 0.04-0.20%. Mean amount of applied dose remaining in the stratum corneum was 0.09% for the high dose and 0.29% for the low dose for the both exposure periods. Mean amount in the remaining epidermis for the 10 hour exposure was 1.71% for the low dose and 0.06% for the high dose. For the 24 hour exposure, mean percent of dose in the epidermis was 1.83% for the low dose and 0.02% for the high dose.

The *in vitro* studies on rat and human skin are Acceptable/Nonguideline supplement and provide supplemental data to the guideline requirement for a dermal penetration study (870.7600).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Note: Results of the *in vivo* rat dermal absorption study are provided for comparison purposes.

Dermal Absorption Rate Summary Mesotrione In Vivo Rat Dermal Absorption Study MRID 46951723								
Dose (µg/cm²)	Mean percentage of Dose Absorbed & In/On Skin							
	10 h		24 h*		72 h		120 h	
	Abs**	Skin***	Abs	Skin	Abs	Skin	Abs	Skin
4.8	0.31	17.62	0.61	17.60	0.96	13.51	1.83	12.60
4781	0.52	0.11	0.16	0.04	0.12	0.03	0.17	0.04

* Abs= radioactivity absorbed (sum of carcass, urine, feces GI tract and cage wash)

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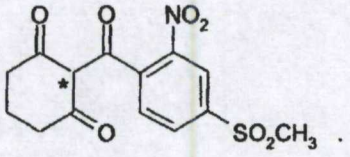
**Amount of radioactivity in/on skin after skin wash (statum corneum and application site skin)

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

[^{14}C]-Mesotrione

Description:	Light beige, solid
Lot/batch #:	Reference No. Y11339/502
Purity:	>95.8% a.i.
Compound stability:	Not available
CAS # for TGAI:	Not available
Structure:	
Vehicle/Solvent used:	Distilled water
Radiolabelling:	Indicated in structure by asterisk
Specific Activity:	1.36 GBq/mM (4.0 MBq/mg)
Radiochemical Purity:	>96.1% (low dose) and 98.9 (high dose) after mixing with unlabeled mesotrione (Ref # Y06684/118)
Source:	Syngenta Crop Protection Inc.
Other comments:	None

2. Relevance of test material to proposed formulation(s): Not relevant

3. Skin membrane source:

Species:	Rat/Human
Strain:	Rat: Wistar Crl: (WI)BR Human: NA
Age/weight at study initiation:	Rat: 28±2 days Human: Not reported
Source:	Rat: Charles River UK Ltd, Margate, Kent, UK Human: surgery or *post-mortem*

B. STUDY DESIGN:

1. Dose:

Rationale: Dose selection was based on direct exposure to formulation concentrate or anticipated dermal deposition of recommended field dilution.

Nominal doses: 480 or 0.504g a.i./L dosing solution.

Actual doses: The actual concentration of [^{14}C]-mesotrione in the dosing solutions were 482 or 0.49 g/L (see Table 1 for details). Actual doses were calculated as the mean dose applied to each of 6 dermal membranes/dose rate/exposure period (10 or 24 hours) for both rat and human skin as determined by radiochemical analysis of the two dosing solutions as close to the time of application as practicable.

Dose volume: 10µL/cm^2 skin (2.54 cm^2/membrane)

Duration of exposures (time from dose to termination): 10 or 24 hrs.

Termination periods (10 or 24 hrs): For diffusion cells exposed for 10 hrs/dose/species (rat or human), receptor fluids were not sampled until the membranes had been exposed to [^{14}C]-mesotrione for 10 hrs. For diffusion cells exposed to [^{14}C]-mesotrione for 24 hrs, samples of receptor fluid were taken at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hrs. The same volume of receptor fluid was replaced with fresh receptor fluid after each sample was taken. After the final receptor fluid sampling, the receptor fluids in all diffusion cells were discarded and the membranes and diffusion cells were subjected to mass balance procedures.

Number of membranes /group: Six membranes were tested in individual diffusion cells/dose group/exposure period.

2. **Membrane preparation:** Rat skin membranes were prepared by carefully shaving the fur from the dorsal and flank region using animal clippers, without damaging the skin. The clipped area was excised and subcutaneous fat removed. The skins were soaked in 1.5M sodium bromide for about 20 hours and rinsed in distilled water. The epidermis was peeled from the dermis and the epidermal membranes were stored frozen until used. Human skin membranes were prepared using skin obtained from surgery or post mortem. The skins were washed in warm distilled water for 40-45 seconds and the epidermis removed from the dermis. The epidermal membranes were stored frozen until used. Prior to use membranes were tested for integrity by measuring the electrical resistance across the membranes. Membranes with a measured resistance of <2.5kΩ (rat) or <10kΩ (human) were discarded.

3. **Dose preparation, application to membranes and quantification:**

Preparation: Dosing suspensions were prepared on the day of dosing or as close as practicable. The formulation concentrate was prepared by mixing sufficient unlabelled mesotrione (3859mg Y06684/118) and [^{14}C]-mesotrione (10 mg mesotrione/36.6MBq; Y11339/5020) in acetonitrile. The solution was mixed thoroughly, dried by rotary evaporation and milled to a particle size of 4.9 microns. This dried powder was mixed with 5635mg of blank formulation (Y11339/503) and mixed thoroughly to ensure homogeneity. The 1/952 aqueous dilution (spray formulation) of the formulation concentrate was prepared by first reducing the appropriate amount of [^{14}C]-mesotrione in acetonitrile (4.86 mg of Y11339/502 mesotrione containing 19.5MBq of radioactivity) to dryness under nitrogen. To this was added 7.40mg blank formulation (Y11339/503), 20.1 mg Agral 90 (Y01105/010) and 9970mg water. The preparation was mixed thoroughly to ensure homogeneity.

Assembly of diffusion cells and application of dosing solutions: Glass diffusion cells were used with an exposed membrane area of 2.54 cm^2. Approximately 3.3cm diameter discs from at least two human subjects or three rats were mounted, dermal side down, in diffusion cells held together with clamps and placed in a water bath at 32 ± 1EC. Cells were prepared such that 6 membranes from at least two subjects/species/dose/exposure period were tested. The receptor chambers of each cell contained a small magnetic stirrer bar and each was filled with a measured volume of 50% ethanol in water. The receptor fluid ensured that the test

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substance could freely partition from the skin to the fluid without reaching a concentration that would limit diffusion. A pre-treatment sample (100µL) was taken from each chamber and analyzed for radioactivity by LSC and an equal volume of fresh receptor fluid was added back. The exposed surface of each membrane was treated with 25.4µL of formulation concentrate or 1/952 spray dilution of the formulation concentrate as given in Table 1.

Quantification: Radioactivity (dpm) and radiochemical purity were determined before application of the dosing solutions to membranes. Radiochemical purity was determined by TLC analysis of the dosing preparations. Homogeneity of the doses was determined from radiochemical analysis of dose-size aliquots of the dosing solutions taken before, during and after dosing. Data are summarized in Table 1.

Table 1. Dose levels achieved

Dose	Application rate (µL/cm^2)	Actual mesotrione concentration (g/L)	Applied dose (µg mesotrione/cell)	Applied dose (MBq/cell)
Concentrate	10	482	12244	0.12
1/952 v/v	10	0.49	12.3	0.05

4. **Sample collection and preparation:** For the 10 hour exposure period, cells were not sampled until 10 hours after application. For the 24 hour exposure period, receptor fluid in the cells were sampled at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours after dosing the membranes. After the final samples were taken the remaining receptor fluids were discarded and the chambers washed with fresh receptor fluid which was also discarded. Material balance for each chamber was performed by first washing the chambers with acetonitrile and analyzing the washings for [^{14}C]-mesotrione by LSC. Then the epidermal surfaces of the membranes were decontaminated by swabbing the application sites with natural sponges pre-wetted with soap solution (3% Teepol®) and with additional sponges wetted with distilled water. Skin surfaces were checked with a Geiger counter for residual radioactivity. Rat skin membranes were too fragile for tape stripping and were solubilized and digested in Soluene 350® without further treatment. Human skin membranes were treated the same except penetration through the stratum corneum was determined by tape stripping successive layers with Scotch tape (Scotch 3M Magic Tape).using a maximum of 5 strips. Radioactivity was extracted from the tape strips with acetonitrile. The remaining human skin was digested with Soluene 350® the same as rat skin membranes.

5. **Sample analysis:** Material balance samples, receptor fluid samples, and aliquots of dosing solutions were analyzed using Liquid Scintillation Counting (LSC) techniques. The LSC techniques were incompletely described. Apparently Optiphase 'High Safe' 3 was the scintillant of choice and 100µL was the preferred sample volume counted. The type of liquid scintillation counter, method of standardization, quench correction and replicates counted were not reported. The limit of quantitation (LOQ) was set at 1.03µg/mL for the formulation concentrate and at 0.003µg/mL for the 1/952 v/v aqueous solution. Total amounts of radioactivity in samples were reported both as µg equivalents of mesotrione and as percentages of the applied dose.

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II. RESULTS:

A. MATERIAL BALANCE AND MESOTRIONE DISTRIBUTION:

The material balance for dermal penetration of mesotrione through rat and human epidermis is shown in Tables 2 and 3, respectively. Recovery of radioactive material was excellent with means ranging from 98.5-103% of the applied dose for rats and 94.6-102% for humans. For the formulation concentrate, skin washing removed the majority of the applied dose from the skin surface after 10 or 24 hours exposure. For rat skin dosed with the formulation concentrate, washing removed 99.4 and 98.2%, respectively and 73.2 and 75.2% for the 1/952 spray dilution. Washing removed 100 and 102% of the applied dose of concentrate at 10 and 24 hours, respectively, for human skin and 92.8 and 91.9% for the 1/952 spray dilution. The residual radioactivity remaining with the epidermis for the concentrated dose applied to rat skin was 0.36 and 0.32% for the 10 and 24 hour exposures, respectively, and for the 1/952 spray dilution was 17.6 and 20.9%, respectively, for the 10 and 24 hour exposures. For the human epidermal applications of the formulation concentrate, 0.06 and 0.02% remained with the epidermis at 10 and 24 hours post-application and 1.71 and 1.83% of the 1/952 spray dilution remained with the epidermis. In addition a portion of the applied doses remained in the stratum corneum of human skin. For the concentrated dose the values were 0.09 and 0.09% at 10 and 24 hours post-exposure and for the 1/952 dilution were 1.71 and 1.83%, respectively. Only 0.02-0.04% of the applied dose remained in the diffusion chambers for the concentrated applications and 0.33-0.95% remained for the dilute applications.

The portion of the applied dose which was absorbed by (penetrated into receptor fluid) rat and human skin is discussed in the following section.

TABLE 2: Material balance for *in vitro* dermal penetration of mesotrione through rat epidermis expressed as percentage of the applied dose

Matrix analyzed	10 hr Exposure		24 hr Exposure	
	Formulation concentrate	1/952 v/v spray dilution	Formulation concentrate	1/952 v/v spray dilution
Donor chamber	0.03±0.02	0.95±0.46	0.04±0.02	0.33±0.05
Skin wash	99.4±0.65	73.2±4.54	98.2±1.44	75.2±3.96
Epidermis	0.36±0.13	17.6±1.84	0.32±0.13	20.9±2.58
Absorbed	<0.05	6.78±1.56	0.05±<0.01	6.59±1.75
Total recovered	99.9±0.58	98.5±3.13	98.6±1.44	103.0±2.45

Data taken from Tables 2 and 3, pp 25 and 26; MRID 46951721.

TABLE 3: Material balance for *in vitro* dermal penetration of mesotrione through human epidermis expressed as percentage of the applied dose				
Matrix analyzed	10 hr Exposure		24 hr Exposure	
	Formulation concentrate	1/952 v/v spray dilution	Formulation concentrate	1/952 v/v spray dilution
Donor chamber	0.02±<0.01	0.41±0.16	0.02±<0.01	0.36±0.13
Skin wash	100±1.37	92.8±1.86	102±0.71	91.9±2.07
Stratum corneum	0.09±0.01	0.29±0.03	0.09±<0.01	0.29±0.04
Remaining epidermis	0.06±0.03	1.71±0.67	0.02±0.01	1.83±1.06
Absorbed	<0.04	0.15±0.02	<0.04	0.20±0.05
Total recovered	100±1.38	95.3±1.60	102±0.70	94.6±1.24

Data taken from Tables 2 and 3, pp 25 and 26; MRID 46951722.

B. TOTAL ABSORBED DOSE:

Absorbed dose data for rat and human skin membranes are given in Tables 4 and 5, respectively. The absorbed dose is expressed as the percentage or amount ($\mu g/cm^2$) of [C^{14}]-mesotrione recovered in the receptor fluid at the specified time or time period. For the concentrate, mesotrione absorption through rat epidermis was fastest between 0 - 6 hours (0.29 $\mu g/cm^2$/hr) of application, slowing to 0.04 $\mu g/cm^2$/hr during the 6-24 hr time period. Between 0-24 hrs, the mean rate of absorption was 0.09 $\mu g/cm^2$/hr. The amounts absorbed during simulated work days of 6, 8, and 10 hours were <1.94, 2.23, and 2.30$\mu g/cm^2$, respectively. The respective amounts expressed as percentages of the applied dose were <0.05, 0.05, and 0.05%. The amount absorbed over the entire 24 hour exposure period was 2.60$\mu g/cm^2$ (0.05% of the applied dose). [^{14}C]-mesotrione absorption through human epidermis was below the limit of quantitation (0.08$\mu g/cm^2$/hr) for the entire 24 hour exposure period (Table 4).

For the 1/952 v/v aqueous dilution, mesotrione absorption through rat epidermis was fastest between 0-1 hours (0.14$\mu g/cm^2$/hr) of application, slowing to 0.01$\mu g/cm^2$/hr between 1-24 hours. Between 0-24 hours, the mean rate of absorption was 0.01$\mu g/cm^2$/hr. The amounts absorbed during simulated work days of 6, 8, and 10 hours duration were 0.23, 0.25, and 0.26$\mu g/cm^2$, respectively (4.79, 5.07, and 5.36%, respectively). The amount absorbed over the entire 24 hour exposure period was 0.32$\mu g/cm^2$ (6.59% of the applied dose). For the 1/952 v/v aqueous dilution, mesotrione absorption through human epidermis was fastest between 0-2 hours (0.0025$\mu g/cm^2$/hr) of application, slowing to 0.0002$\mu g/cm^2$/hr) of application, slowing to 0.0003$\mu g/cm^2$/hr between 2-24 hours. Between 0-24 hours, the mean rate of absorption was 0.0003$\mu g/cm^2$/hr. The amounts absorbed during simulated work days of 6, 8, and 10 hours duration were 0.007, 0.008, and 0.008$\mu g/cm^2$, respectively (0.15, 0.16, and 0.16%, respectively). The amount absorbed over the entire 24 hour exposure period was 0.01$\mu g/cm^2$ (0.20% of the applied dose) as given in Table 5.

TABLE 4. Summary of *in vitro* absorption of mesotrione through rat epidermis					
Dose Group	Mean absorption rates		Mean amount and percent of dose absorbed		
	Time period (hrs)	Absorption rate ($\mu g/cm^2/hr$)	Time (hrs)	Amount ($\mu g/cm^2$)	Percent absorbed
Concentrate (482g mesotrione/L) n=6	0-6	0.29±0.06	6	<1.94	<0.05
	6-24	0.04±0.01	8	2.23	0.05
	0-24	0.09±0.01	10	2.30	0.05
			24	2.60	0.05
			LOQ	1.93	0.04
1/952v/v aqueous spray dilution n=6	0-1	0.14±.0.04	6	0.23	4.79
	1-24	0.01±<0.01	8	0.25	5.07
	0-24	0.01±<0.01	10	0.26	5.36
			24	0.32	6.59
			LOQ	0.004	0.86

Data taken from Table 1, page 24; MRID 45951721.

TABLE 5 Summary of *in vitro* absorption of mesotrione through human epidermis					
Dose Group	Mean absorption rates		Mean amount and percent of dose absorbed		
	Time period (hrs)	Absorption rate ($\mu g/cm^2/hr$)	Time (hrs)	Amount ($\mu g/cm^2$)	Percent absorbed
Concentrate (482g mesotrione/L) n=6	0-24	<0.08	6	<1.84	<0.04
			8	<1.84	<0.04
			10	<1.84	<0.04
			24	<1.84	<0.04
			LOQ	1.83	0.04
1/952v/v aqueous spray dilution n=5	0-2	0.0025±0.0007	6	0.007	0.15
	2-24	0.0002±0.0001	8	0.008	0.16
	0-24	0.0003±0.0001	10	0.008	0.16
			24	0.010	0.20
			LOQ	0.005	0.10

Data taken from Table 1, page 24; MRID 45951722.

Given the uncertainty regarding actual deposition under actual field conditions, it is considered appropriate to derive an estimate of dermal absorption based on the results from the low dose group (1/952 spray dilution of the formulation concencrate, as percent dermal absorption was greatest at this dose level. Based on the likely worker exposure time frame, it is considered most appropriate to adopt the dermal absorption value calculated for this dose rate both for rat and human skin in this *in vitro* study. The radioactivity present in the stratum corneum of the human skin at termination is considered not to be available for further absorption but to be labile to loss by desquamation.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

The results obtained in the two combined *in vitro* studies (MRID Nos 46951721 and 46951722) indicate that mesotrione is absorbed through rat epidermis from the concentrate formulation and 1/952 v/v aqueous dilution, at a very slow rate and through human epidermis at an extremely slow rate. The majority of the applied dose (between 73.2-99.4% for rat skin and 91.9-102% for human skin) was removed by mild skin washing at 10 and 24 hours post-treatment. Higher proportions were associated with the epidermis when mesotrione was

applied as the spray dilution, compared with the concentrate. However, in terms of actual amounts absorbed ($\mu g/cm^2$), these values were very small. These data predict that the human dermal absorption of mesotrione from potential exposure to the SC formulation (A12738A), either as the formulation concentrate or as a 1/952 v/v aqueous spray-strength dilution, would be low (rat epidermis) to minimal (human epidermis).

B. REVIEWER COMMENTS:

This study was carried out to determine the *in vitro* percutaneous absorption of mesotrione through rat and human epidermis following exposure to a [C^{14}]-mesotrione formulation concentrate or a 1/952 v/v aqueous spray dilution of the [C^{14}]-mesotrione formulation including 0.2% Agral 90 adjuvant. The former was included to assess exposure to mixer/loaders and the latter to simulate exposure of spray applicators in the field. Exposure periods of 10 and 24 hours were incorporated. The experiments were carried out using diffusion chambers with rat or human epidermal membranes prepared from skin obtained by excising shaved skin from rats or from human skin obtained during surgery or post-mortem. The doses of formulation concentrate or spray dilution were applied to the intact rat or human epidermal membranes and allowed to diffuse freely into receptor fluid contained in the diffusion cells. Receptor fluid was sampled once for the 10 hour exposures to determine total absorption during a simulated work day and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hrs for the 24 hour exposures to determine the rate of absorption through the epidermis. Total radioactivity recovered from the chambers, skin wash, epidermal membranes and receptor fluids was excellent, ranging between 98.6-102% for the formulation concentrate and 94.6-103% for the 1/952 aqueous dilution of the formulation concentrate. The amount absorbed ranged between <0.04 - <0.05% of the applied for the concentrate and 0.15 - 6.78% for the spray dilution. The absorption data for the formulation concentrate and spray strength dilution indicated that mesotrione was very poorly absorbed through rat and human skin *in vitro* and the rates of absorption were very slow for rat epidermis and extremely slow for human epidermis.

The *in vitro* studies on rat and human skin are Acceptable/Nonguideline supplement and provide supporting data to the guideline requirement for a dermal penetration study (870.7600).

C. STUDY DEFICIENCIES:

Some liquid scintillation procedures and sample preparation methods were incompletely described. Because material balances were high for both dose rates at each sampling interval, these omissions did not appear to affect the results of this *in vitro* dermal penetration study using membranes prepared from rat and human skin.